OPERATING INSTRUCTIONS

510 Meta inverted confocal

You must not operate this equipment without prior training from a BALM facility staff member.

To arrange training and for help please contact:

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Standard Operating Procedure

How to turn the equipment on:

1. Switch on the metal halide lamp
2. Switch on the Zeiss remote control
3. Switch on the computer and log in

How to turn the equipment off:

1. Switch off lasers and wait to cool
2. Switch off Zeiss remote control once lasers have cooled
3. Switch off computer
4. Switch off metal halide lamp

Rules of use:

This microscope should be treated with respect and care at all times.

This Microscope can only be used by Masters by Research or PhD students, Postdocs and members of staff.

The microscope lenses must be cleaned after every usage and the equipment treated carefully at all times.

If you have any problems at all with the microscope, no matter how trivial they may seem please see a technician immediately.

REMEMBER: You have 5GB of disk space on this microscope. Check before you start if you have room for your experiment. If not, delete your old data.

1) Open **ZEN** software
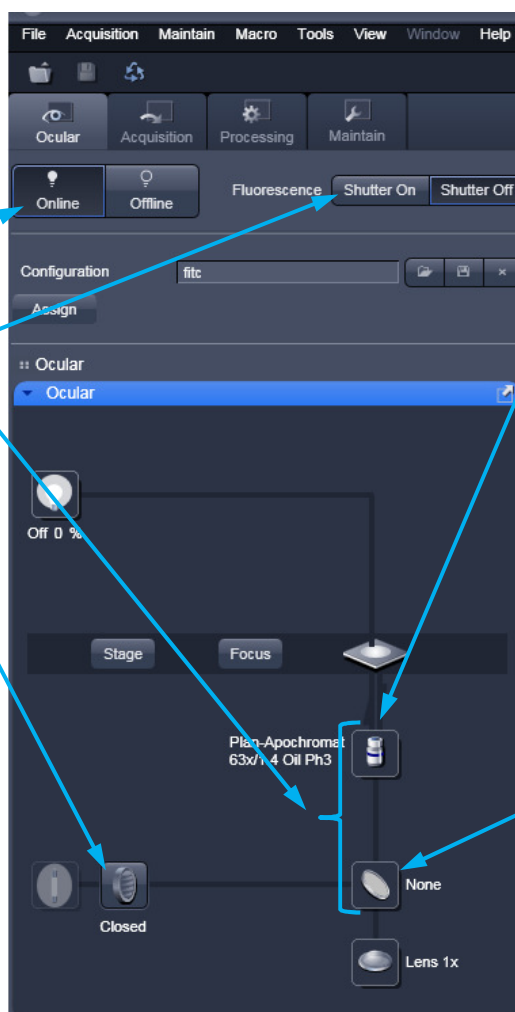
2) Click **Start System**

3) Place slide on microscope stage

4) In the **Ocular** tab choose an objective and a filter

5) Click **Online** and open the shutter

6) Find your sample

Use this button to change the objectives

Objectives available:

    10x, 20x – air
    40x, 63x, 100x – oil

Automated, do not move by hand

NB: please LOWER THE STAGE before changing between objectives (to avoid crashing lens onto slide)

Use this button to change between the fluorescent filters

7) In the **Acquisition** tab
   select a **Configuration**
   and accept the prompt asking if you wish to
   switch on the lasers
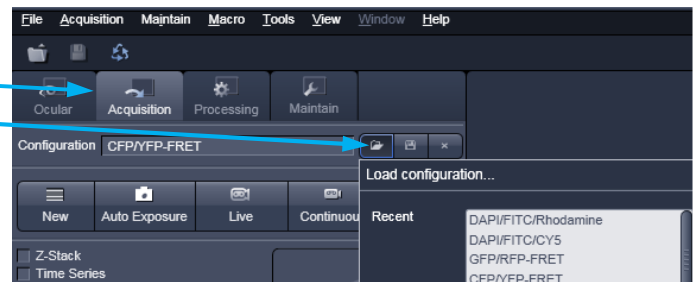
8) In the **Laser** menu
   Switch the Argon laser from **standby** to **on**

9) In the **laser properties** (for the Argon laser)
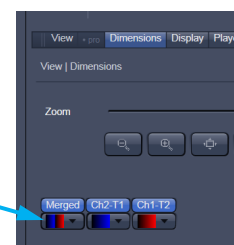   Set the **tube current** to 6.1A by adjusting the
   output (%)

   NB. Ignore the warning about exceeding 50% output

10) Click **Auto Exposure** to generate an
    initial image

11) Click **Live** to see the live image, and select the
    SPLIT view to see the individual channels
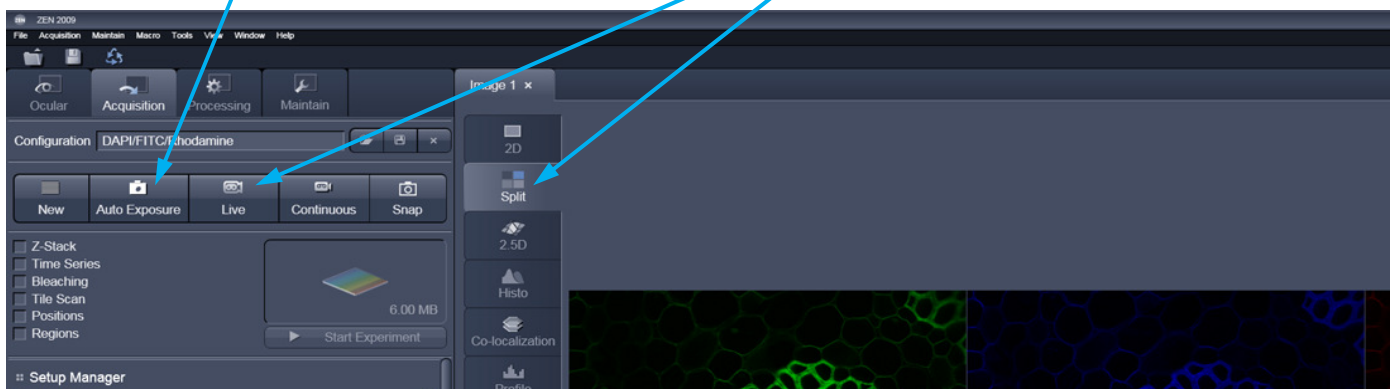
12) Optimise the system by clicking **Merged**

Blue = Underexposed pixels
Red = Overexposed pixels
Adjust the **Gain** and **Digital Offset** so that the signal is within the dynamic range of the detectors (see below)

13) In the **Channels** menu click **Show all** and **Select all**
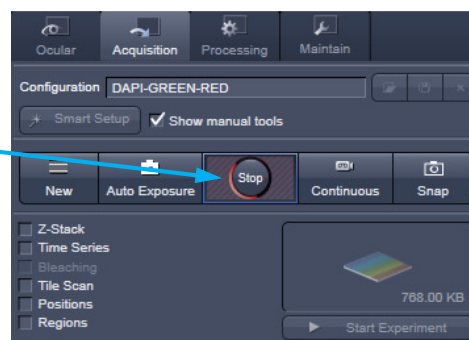
14) Set the pinhole to **1AU**

15) Alter the **Digital offset** so background is black not blue

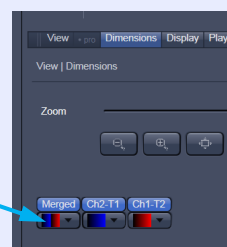16) Alter the **Gain (Master)** so there are no red pixels

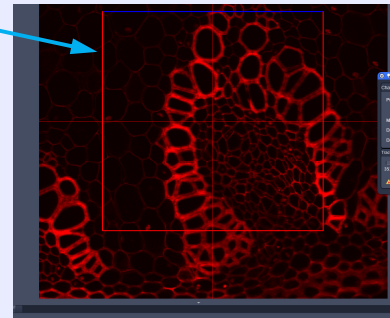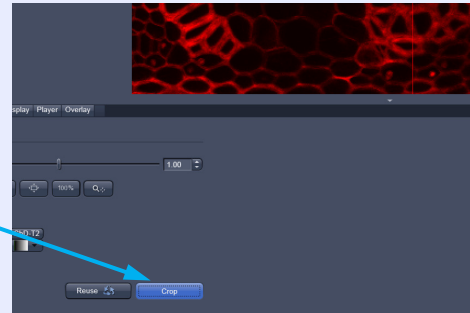17) Repeat for each channel

18) **Stop** the Live image aquisition

Click the **Merged** button again to return to the original colours

You can **Crop** your image
and change the position, size or rotation of the cropped area
by moving the crop box

Start scanning again and your cropped image will appear

19) In the **Acquisition Mode** menu
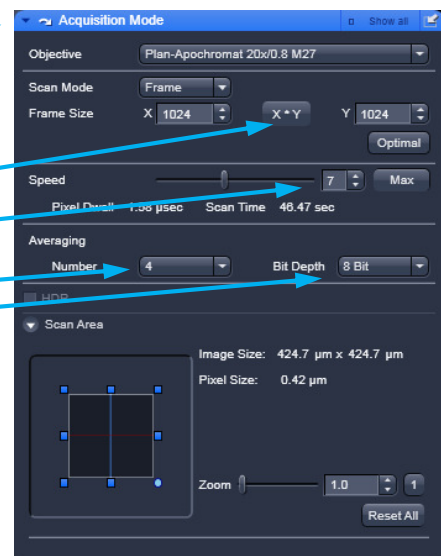   set the image capture parameters

Recommended acquisition:
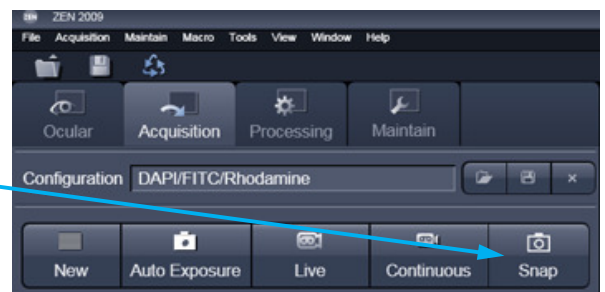Frame Size: **1024 x 1024**
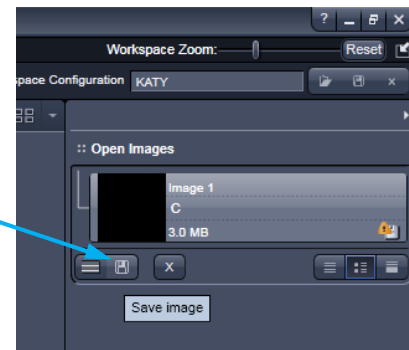Speed: **7**
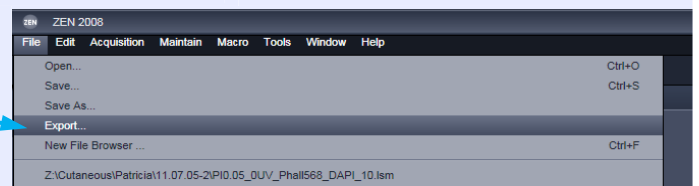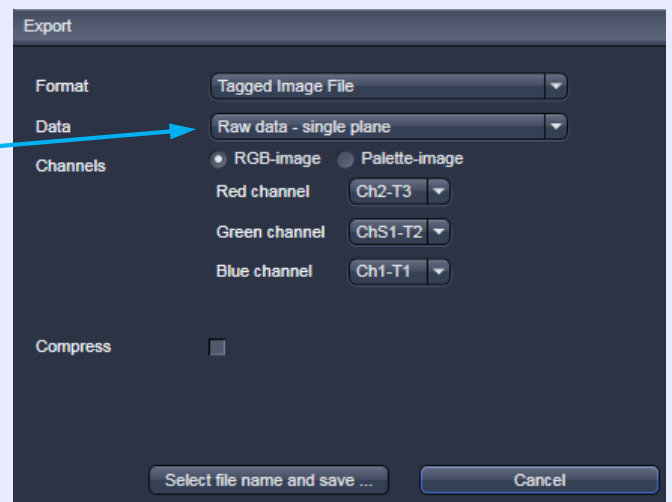Averaging number: **4**
Bit depth: **8 bit**

20) Click **Snap**

21) Save your image on the **E: drive**

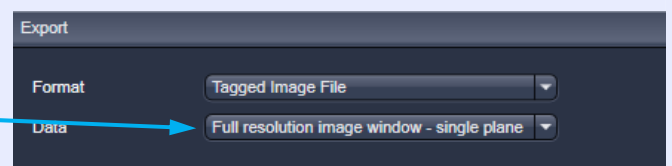(displayed in the top right of the workspace)

To export your images in TIF format, go to **File,** and **Export.**

Export as **Raw data – single plane** for overlay image.

Export as **Full resolution image window** for contents of the image window (will vary depending on which tab is open).

When you have finished, transfer all your data to the **Z network drive**

PLEASE TIDY UP!!
Clean lenses, throw away used tissue/lens tissue, dispose of old slides in the yellow sharps bin